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Review Article

Cucurbits: Potential Suppliers of Antioxidants

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Abstract: One of the necessities of a healthy life is to have adequate amounts of antioxidants in the body and neutralize the damage caused by free radicals. Free radicals are continuously produced in the human body, as they are essential for energy supply, detoxification, chemical signaling and immune function but their over-production due to exposure to external oxidant substances or a failure in the defense mechanisms, increases risk of different disease. Powerful antioxidants perform multiple functions essential for good health and help organisms deal with oxidative stress, caused by free radical damage. Natural production of antioxidants in body decreases with time and age, so it becomes essential to include antioxidant rich food items in the diet. In the recent years, in the attempt to counteract the detrimental effects of oxidative damages is always more convincing the strategy of implementing the diet with antioxidants nutrients, especially deriving from natural sources. Literature survey illustrates that cucurbits like gourd, bitter gourd and cucumber are potential source of antioxidants. Thus considering the growing interest in assessing the antioxidant capacity of vegetables an attempt has been made to undertake this study to evaluate the potential of commonly edible cucurbits, as vegetables especially in Maharashtra, as a dietary suppliers of antioxidants by using the standard methods. A comparative and quantitative analysis would lead to determine the significant importance and ranking of various edible cucurbits. In the current study, Tondli has proved to be a very good dietary supplement owing to its high phenolic content, flavonoid content and reducing ability.

Keywords: Antioxidants, assays, cucurbits, flavonoids, free radicals

Introduction

An antioxidant is a molecule which inhibits the oxidation of other molecule. Cells may suffer from oxidative stress if levels of antioxidants are low. Plants make use of different antioxidants such as flavonoids, phenols, catalase, and superoxide dismutase[1]. Reports suggest that many phytochemicals are being increasingly explored for their antioxidant property[2]. One of the necessities of a healthy life is to have adequate amounts of antioxidants in the body and neutralize the damage caused by free radicals[3,4]. Free radicals are continuously produced in the human body, as they are essential for energy supply but their over-production due to exposure to external oxidant substances or a failure in the defense mechanisms, increases risk of different diseases[5].

There is an inverse proportion in the uptake of vegetables or fruits with morbidity and motality[6]. Vegetables offer protection against the diseases without the need of supplementary micronutrients[7]. The antioxidant potential of plants is one of the properties for their use as medicine ingredients[8,9]. The antioxidants from plants have greater benefits as compared to synthetic antioxidants because the synthetic antioxidants have been shown to be carcinogenic[10,11]. Phenolic compounds present in vegetables are responsible for antioxidant properties[11]. 10 plants from the Cucurbitaceae family namely *Cucumis sativus L., Cucurbita maxima Duch., Lagenaria siceraria Mol., Momordica charantia L., Coccinia grandis L., Luffa acutangula L., Luffa cylindrica L., Trichosanthes anguina L., Trichosanthes nervifolia L., Praecitrullus fistulosus L.* were used as test samples. Present investigation had indicated the presence of phenolic compounds, reducing and chelating abilities in all tested sample, therefore, these vegetables can be considered as one of the potential sources of antioxidants.

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Materials and Methods

Chemicals:

Methanol, 10% Folin-Cioalteau reagent, 7.5% Sodium carbonate, 200mM Sodium phosphate buffer, 1% Potassium ferricyanide, 10% Trichloroacetic acid, 0.1% Ferric chloride, 500μM Ferrous sulphate, 0.1M Tris-HCl (pH-7.8), 0.25% 1,10-phenanthroline, 10% Aluminium chloride, 1 Mole/lit sodium acetate, 95% ethanol, 0.1 M phosphate buffer, 43 mM hydrogen peroxide solution.

I] **Vegetable:**

Sample Preparation:

The fresh vegetables were collected from the local market. They were washed with distilled water to remove dirt; the water was drained off and vegetables sun-dried until the water was removed. 1g of pulp sample was weighed and crushed finely with the help of mortar and pestle. 10 ml methanol was added to it. This homogenate was filtered, collected and evaporated to dryness. This was reconstituted with 50 ml distilled water and later used for analysis.

Total Phenol Determination:

The total phenol content was determined by mixing 0.5 ml of the sample extracts with 2.5 ml 10% Folin-Cioalteau reagent (v/v), 2.0 ml of 7.5% sodium carbonate was subsequently added. The reaction mixture was incubated at 45ºC/40 min. Absorbance was measured at 765 nm using a spectrophotometer. Tannic acid was used as standard phenol[12].

Determination of Reducing Property:

Appropriate dilutions (0-1.0 ml) were mixed with 2.5 ml of 200 mm sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide was added to it. The mixture was incubated at 50ºC for 20 mins. Thereafter, 2.5 ml of 10% trichloroacetic acid was added and subsequently centrifuged at 650 rpm for 10 mins. 5 ml of the resulting supernatant was mixed with equal volume of water and 1 ml of 0.1% ferric chloride. The absorbance was taken at 700nm against a reagent blank[13].

Fe^{2+} Chelation Assay:

Briefly 150 µl of freshly prepared 2mMFerrous sulphate was added to a reaction mixture containing 168 µl of 0.1 M Tris-HCL (pH 7.4), 218 µl saline and the methanolic extracts (0-5000µl). The reaction mixture was incubated for 5 min; before the addition of 13µl of 0.25% 1, 10-phenanthroline (w/v). The absorbance was subsequently measured at 510 nm in the spectrophotometer[14].

Estimation of Total Flavonoid Content from pulp:

The standard solution or extract (0.5ml) were mixed with 1.5ml of 95% ethanol (v/v) , 0.1ml of 10% aluminium chloride (w/v) , 0.1 mol/L sodium acetate & 2.8ml distilled water. The volume of 10% aluminium chloride was substituted by same volume of distilled water in blank. After incubation at room temperature for 30mins, the absorbance of the reaction mixture was measured at 415nm[15].

II] **Seed:**

Sample Preparation:

The fresh seeds were collected from Pune market. They were washed with distilled water to remove dirt; the water was drained off and seeds were powdered. 1g of sample was weighed and 10 ml methanol was added. This homogenate was then filtered, collected & evaporated to dryness. This was reconstituted with 50 ml distilled water & later used for analysis.

Estimation of Total Flavonoid Content of the seed:

The standard solution or extract (0.5 ml) were mixed with 1.5ml of 95% ethanol (v/v), 0.1 ml of 10% aluminium chloride 42 (w/v) , 0.1 mol/L sodium acetate & 2.8 ml distilled water. The volume of 10% aluminium chloride was substituted by same volume of distilled water in blank. After incubation at room temperature for 30 mins, the absorbance of the reaction mixture was measured at 415nm[15].

Result

I] **Vegetable:**

Total Phenol Determination:

The total phenol content of the methanolic extracts of 10 test vegetables are given in table I. The highest phenolic content was found in *Lagenaria siceraria Mol.* (121 µg/g) followed by *Coccinia grandis L.* (115 µg/g). *Momordica charantia L.* and *Luffa cylindrica L.*also show considerable presence of phenolic content.

Table.I: Total Phenol content (µg/g) of the methanolic extracts

Extracts		Total phenol content (µg/g)
Botanical Name	**Local Name**	
Cucumis sativus L.	Cucumber	47
Cucurbita maxima Duch	Red Bhopla	57
Lagenaria siceraria Mol.	White Bhopla	121
Momordica charantia L.	Karla	76
Coccinia grandis L.	Tondli	115
Luffa acutangula L.	Dodka	59
Luffa cylindrica L.	Ghosavla	62
Trichosanthes anguina L.	Snake Gourd	44
Trichosanthes nervifolia L.	Parwal	52
Praecitrullus fistulosus L.	Tinda	42

Determination of Reducing Property:

The reducing power of the 10 test vegetables is illustrated in the figure I. *Cucumis sativus L.* shows highest reducing power among all the test vegetables. The reducing power of *Coccinia grandis L.* and *Luffa cylindrica L.* is also good making them good antioxidants.

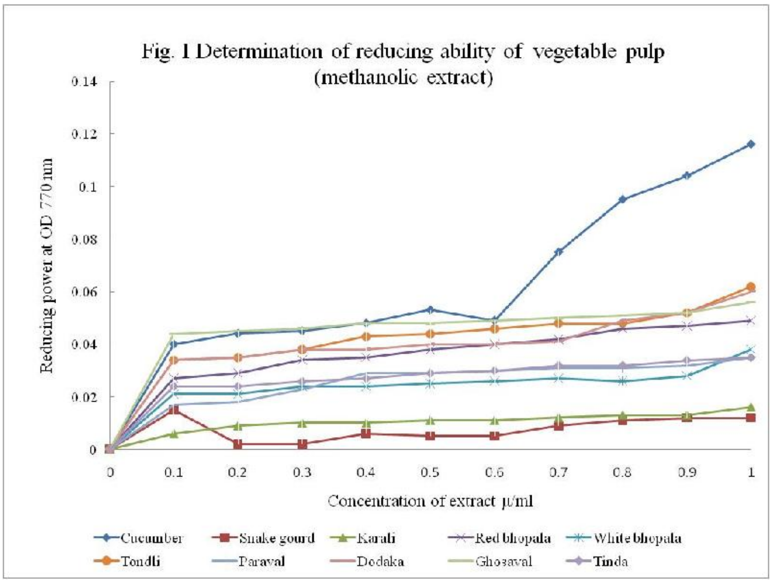

Fig. I Determination of reducing ability of vegetable pulp (methanolic extract)

Fe^{2+} Chelation Assay:

The iron chelating abilities of the 10 test vegetables is given in figure II. Ability of chelating Fe^{2+} is one of the properties of antioxidants. Maximum iron chelating capacity was displayed by *Lagenaria siceraria Mol.* followed by *Coccinia grandis L.* and *Cucurbita maxima Duch*.

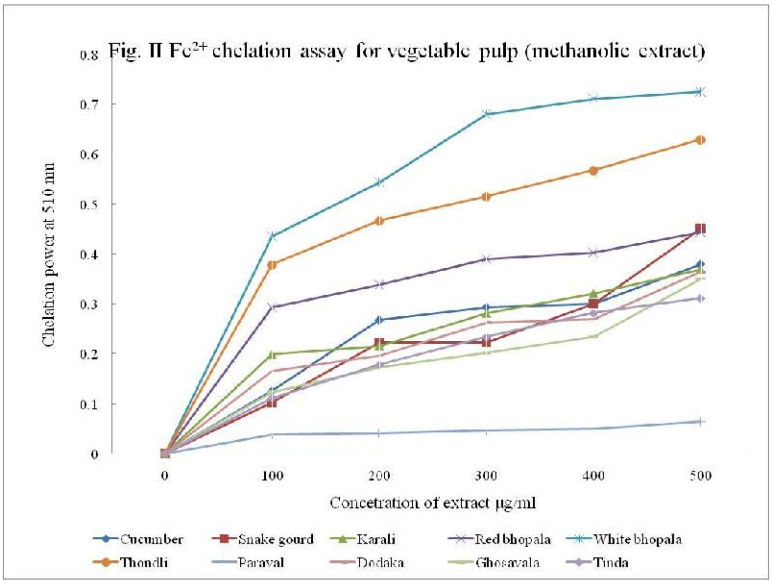

Fig. II Fe^{2+} chelation assay for vegetable pulp (methanolic extract)

Estimation of Total Flavonoid Content from pulp:

The total flavonoid content of the vegetable pulps is given in table II. Highest flavonoids content was obtained in *Coccinia grandis L.* (89 μg/g) followed by *Lagenaria siceraria Mol.* (70 μg/g). In addition to these two vegetables, *Praecitrullus fistulosus L.* and *Trichosanthes anguina L.* are also found to contain substantial amount of flavonoids.

Table.II: Total Flavonoid content (μg/g) of the methanolic extracts from Pulp

Extracts		Total flavonoid content (μg/g)
Botanical Name	**Local Name**	
Cucumis sativus L.	Cucumber	63
Cucurbita maxima Duch	Red Bhopla	47
Lagenaria siceraria Mol.	White Bhopla	70
Momordica charantia L.	Karla	78
Coccinia grandis L.	Tondli	89
Luffa acutangula L.	Dodka	89
Luffa cylindrica L.	Ghosavla	55
Trichosanthes anguina L.	Snake Gourd	83
Trichosanthes nervifolia L.	Parwal	65
Praecitrullus fistulosus L.	Tinda	83

II] **Seed:**

Estimation of Total Flavonoid Content

The total flavonoid content of the seed samples is given in table III. Highest flavonoids content was obtained in *Coccinia grandis L.* (29 μg/g) followed by *Cucurbita maxima Duch* (28 μg/g). Although the pulp contains higher amounts of flavonoids, the seeds are also potential candidates having antioxidant activities.

Table.III: Total Flavonoid content (µg/g) of the methanolic extracts from seed

Extracts		Total flavonoid content (µg/g)
Botanical Name	Local Name	
Cucumis sativus L.	Cucumber	06
Cucurbita maxima Duch	Red Bhopla	28
Lagenaria siceraria Mol.	White Bhopla	10
Momordica charantia L.	Karla	11
Coccinia grandis L.	Tondli	29
Luffa acutangula L.	Dodka	09
Luffa cylindrica L.	Ghosavla	22
Trichosanthes anguina L.	Snake Gourd	09
Trichosanthes nervifolia L.	Parwal	12
Praecitrullus fistulosus L.	Tinda	07

Discussion

The present analysis of the 10 vegetables from the Cucurbitaceae family shows the various components like phenols, flavonoids which determine their antioxidant potential. There was corresponding increase in the reducing ability with increase in concentration of the extracts, indicating a dose-dependent relationship. Cucumber had showed highest reducing power (Fig. I), whereas White bhopala followed by Tondli showed high chelating ability of Fe2+ (Fig. II).

The structure and substitution pattern of hydroxyl groups in flavonoids is responsible for their antioxidant activity[10]. Around 3000 flavonoids have been described to be obtained from plant kingdom[2]. Phenolic compounds have attracted the interest of researchers because they show promise of being powerful antioxidants that can protect human body from free radicals. The higher is the property the higher is the antioxidant activity. Our analysis demonstrated that the tested vegetables are really important source of antioxidant[7]. The reducing power of vegetable extract was potent and the power of the extract was increased with quantity of sample.

Antioxidants are our first line of defense against free radical damage, and are critical for maintaining optimum health and wellbeing. The need for antioxidants becomes even more critical with increased exposure to free radicals. As part of a healthy lifestyle and a well-balanced, wholesome diet, antioxidant supplementation is now being recognized as an important means of improving free radical protection. Studies on antioxidant interaction with the free radicals will enable us to know the effects of oxidative stress in both normal physiologies as well as in diseased states[1]. The cold-stress to plants induces high levels of reactive oxygen species and hydrogen peroxide. Antioxidants play an important role in curbing down the overproduction of such reactive species[16]. The dietary plants with high antioxidant capacities can help in induction of phase 2 or detoxification enzymes which are more readily capable of neutralizing the toxic/ carcinogenic agents[7]. Many reports of use of plants as medicines in treatment of various diseases have been given. Pumkin, known for its antioxidant potential is being used against various human diseases like benign prostatic hyperplasia (BPH), prostrate cancer, stomach cancer, cadmium toxicity, pulmonary ailments[17]. *Z. alatum* fruit extracts have been considered to work against various oxidant stresses owing to their antioxidant activity[14]. Roots of *M. citrifolia* contain high levels of phenols and flavonoids and thus can be considered as potent medicines in treatment of human diseases[11]. Pericarp of *Cucurbita maxima.* Duch. Ex Lam has been found effective against prostrate problems[18]. The fractions of turmeric oil have been used against the mutagenicity of sodium azide due to its antioxidant potential. Polysaccharide of Pumkin cell wall also shows antioxidant potential. This study noted that the processed pumkin food can have high antioxidant levels if the polysaccharide composition and structure is effectively investigated while processing[19]. The antioxidant potential of our test samples has made them candidate medicines for prevention and treatment of various human diseases.

Conclusion

- Both pulp and seed of Cucurbits have potent antioxidant with the pulps exhibiting higher antioxidant activity than seeds.
- *Coccinia grandis L.* (Tondli) shows very good phenol content, flavonoids content, reducing power as well as ability to chelate Fe^{2+}.

- It can be recommended from the current study that tondli is a very beneficial dietary supplement for a healthy life.
- Other vegetables like *Lagenaria siceraria Mol.* (White bhopla), *Cucumis sativus L.* (Cucumber) and *Cucurbita maxima Duch* (Red bhopla) which show good antioxidant properties should also be included in daily diet in order to prevent diseases.

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